were treated with H-89, a PKA inhibitor, W-7, a calmodulin inhibitor or LY294002, a PI3K inhibitor prior to 4α-PDD stimulation in SOX9-dependent reporter assay. To examine the chondrogenic differentiation, ATDC5 cells were co-stimulated with 0 to 120 nM 4α-PDD and 10 μg/ml insulin for 10 day, then cells were stained with alcian blue. The amount of SOX9 protein was estimated by Western Blot analysis using anti-SOX9 antibody.
Results: We have screened 120,000 cDNA clones and identified 46 genes that activated SOX9-dependent reporter activity. In cDNA microarray analysis, the mRNA levels of Sox5, Sox6, Ifitm5, Myd116, Mef2c and TRPV4 genes were elevated during chondrogenic differentiation of ATDC5 cells treated with insulin. TRPV4, a cation channel molecule was further investigated in this study since it had a strong effect on SOX9-dependent transcription. mRNA expression of TRPV4 gene was observed in ATDC5, C3H10T1/2, murine primary chondrocytes prepared from the rib cages and hind limb buds in embryonic day12 embryos, but not in NIH3T3. When ATDC5 cells or C3H10T1/2 cells were treated with various concentration of 4α-PDD, SOX9-dependent transcription was elevated in dose dependent manner and this effect was abolished by the addition of ruthenium red, a TRPV antagonist. In ATDC5 cells, H-89 and W-7 inhibited SOX9-dependent reporter activity caused by the stimulation with 4α-PDD while LY294002 did not. When ATDC5 cells were co-stimulated with 4α-PDD and insulin, GAG accumulation was significantly increased as compared with insulin alone whereas 4α-PDD alone showed no effect. Similar result was obtained in C3H10T1/2 cells co-stimulated with 4α-PDD and BMP-2. Co-stimulation with 4α-PDD demonstrated further elevations of mRNAs for type II collagen and aggrecan in ATDC5 cells when compared to insulin alone. 4α-PDD stimulation increased the amount of SOX9 protein in both ATDC5 and C3H10T1/2 cells.
Conclusions: We have identified TRPV4 by its ability to activate SOX9-dependent transcription. Activation of TRPV4 promoted chondrogenic differentiation of ATDC5 cells in cooperation with insulin and C3H10T1/2 cells in corporation with BMP-2 *in vitro*. The protein level of SOX9 was increased by the stimulation with 4α-PDD. These observations suggest that TRPV4 may correlate the process of chondrogenesis.

143 BENEFICIAL EFFECTS OF LIVER X RECEPTOR (LXR) MODULATION ON MATRIX METABOLISM AND PROSTAGLANDIN E_2 (PGE_2) PRODUCTION IN CARTILAGE

Z. Yang[1], N. Li[1], M.A. Rivera-Bermudez[1], S.S. Glasson[1], L.A. Collins-Racie[1], E.R. LaVallie[1], S. Nagpal[2], E.A. Morris[1], C.R. Flannery[1]. *[1]Women's Health & Musculoskeletal Biology, Wyeth Research, Cambridge, MA, USA, [2]Women's Health & Musculoskeletal Biology, Wyeth Research, Collegeville, PA, USA*

Purpose: Osteoarthritis (OA), the most common arthritic condition in humans, is characterized by the progressive degeneration of articular cartilage accompanied by chronic joint pain. Inflammatory mediators, such as cytokines and prostaglandin E_2 (PGE_2) which are elevated in OA joints, play important roles in the progression of cartilage degradation and pain-associated nociceptor sensitivity. LXRs possess potent anti-inflammatory properties, possibly through antagonism of the NFκB signaling pathway. This study was undertaken to evaluate the function of LXRs in cartilage, particularly their capability to inhibit degradation of matrix proteins such as aggrecan, and production of inflammatory and pain mediators such as PGE_2.
Methods: *Mouse cartilage explant culture*: Hip cartilage from wild type and Lxrβ$^{-/-}$ mice were collected and cultured in serum-free medium with or without Il-1β to study the role of Lxrβ in regulating cartilage matrix metabolism and inflammation. Culture medium pooled from wt or Lxrβ$^{-/-}$ explant cultures was analyzed for aggrecanase-generated AGEG aggrecan neoepitope.
Human cartilage explant study: Cartilage explants from human OA joints were treated with/without Il-1β/Oncostatin M +/− cotreatment with LXR agonist GW3965 (10 days) to measure the effect of LXR activation on cartilage matrix degradation and the PGE_2 production. Culture medium was analyzed for aggrecanase-generated AGEG aggrecan neoepitope.
TLDA (Taqman Low Density Array) analysis: A TLDA was designed to include a list of selected genes known to be important in cartilage metabolism and inflammation. Cartilage RNA from 5 human cartilage samples treated with/without Il-1β/Oncostatin M +/− GW3965 was analyzed to determine the effects of GW3965 treatment on the expression of genes such as ADAMTS4, mPGES, TIMP3, MMP-1 and MMP-13.
Results: We have found that both LXRα and β are expressed in cartilage, with LXRβ being the predominant isoform. We show that genetic disruption of Lxrβ gene expression in mice results in significantly increased proteoglycan (aggrecan) degradation and PGE_2 production in articular cartilage treated with Il-1β, indicating a protective role of LXRs in cartilage. Using human cartilage explants, we have found that activation of LXRs by a synthetic agonist significantly reduces cytokine-induced degradation and loss of aggrecan from the tissue. Furthermore, LXR activation dramatically diminished basal level as well as cytokine-induced PGE_2 production by human osteoarthritic cartilage. These effects were achieved at least partly by repression of the expression of ADAMTS4, a physiological cartilage aggrecanase, and microsomal prostaglandin E synthase-1, a key enzyme in the PGE_2 synthesis pathway.
Conclusions: Modulation of LXR signaling in cartilage using small molecule agonists may have great potential for OA therapy, not only to treat symptoms such as pain by blocking PGE_2 synthesis, but also to prevent and possibly reverse disease progression by blocking cartilage matrix degradation.

144 S100A8/S100A9 PROTEINS PLAY A ROLE IN CARTILAGE DEGENERATION IN OSTEOARTHRITIS

H. Zreiqat[1,2], D. Belluoccio[3], M.M. Smith[4], J. Roth[5], T. Vogl[5], J.F. Bateman[3], C.B. Little[4]. *[1]School of AMME and Bosch Institute, University of Sydney, Sydney, 2006, AUSTRALIA, [2]Faculty of Engineering and Bosch Institute, University of Sydney, Sydney, 2006, AUSTRALIA, [3]Murdoch Childrens Research Institute and the Department of Paediatrics, University of Melbourne, Melbourne, AUSTRALIA, [4]Kolling Institute of Medical Research, University of Sydney at Royal North Shore Hospital, Sydney, AUSTRALIA, [5]Institute of Immunology, University of Muenster, Muenster, GERMANY*

Purpose: S100A8 and S100A9 calcium binding proteins are implicated in inflammatory arthritis. Recently S100A8 and S100A9 were shown to be localized in murine and human growth plate and articular cartilage chondrocytes and their expression upregulated by inflammatory cytokines. To-date however, the changes in S100A8, S100A9 and their complex (S100A8/S100A9) in cartilage during the onset and progression of osteoarthritis (OA), and their effect on chondrocyte metabolism have not been examined. This prompted us to determine (1) the *in vivo* expression and immunolocalization of S100A8, S100A9 in cartilage during progressive cartilage destruction in an OA model in mice; (2) the effect of interleukin-1α (Il-1) and retinoic acid (RetA) on S100A8 and S100A9 expression and the immunolocalization of S100A8 and S100A9 in mouse cartilage explants *in vitro*; and (3) the effect of S100A8 and S100A9 on the expression by chondrocytes of key extracellular matrix molecules and matrix degrading enzymes.
Methods: S100A8 and S100A9 *in vitro* protein localization and gene expression were determined in chondrocytes in a surgically induced mice model of OA and in mouse femoral head cartilage explants stimulated with Il-1 or RetA. The effect of recombinant S100A8, S100A9 or the complex on the expression of aggrecan, collagen type II, ADAMTS-1, -4 & -5, MMP-1, -3, -13 & -14 and TIMPs-1, -2 & -3 by primary adult ovine articular chondrocytes was determined using quantitative reverse transcriptase real time polymerase chain reaction (qRT-PCR).
Results: Positive immunostaining for intracellular S100A8 but not S100A9 was found in normal articular chondrocytes in mice. Loss of the chondrocytes S100A8 staining occurred in OA, in contrast to the positive reactivity for both S100A8 and S100A9 in chondrocytes in inflammatory arthritis. However, there was an increase in chondrocyte S100A8 and S100A9 gene expression in early OA which decreased below baseline in late disease. *In vitro*, stimulation with Il-1 but not RetA, increased chondrocyte S100A8 and S100A9 mRNA and protein levels. Homodimeric S100A8 and S100A9, but not the heterodimeric complex, significantly upregulated chondrocyte ADAMTS-1, -4 and -5, MMP-1, -3 and -13 gene expression, while collagen type II and aggrecan mRNAs were significantly decreased.
Conclusions: Our data suggests that intracellular S100A8, but not S100A9, has a physiological role in normal articular chondrocytes in mice. However, the release of homodimeric S100A8 or S100A9 into the pericellular microenvironment, where they may act as cytokine-like molecules upregulating MMPs and aggrecanases while decreasing aggrecan and collagen mRNA expression, may contribute to cartilage degradation in OA. As extracellular S100A8/S100A9 complex does not regulate chondrocyte genes, an imbalance in regulation of the individual S100A8 or S100A9 proteins maybe important in this disease. New efforts to prevent the progression of OA might include strategies that target increased chondrocytes S1008 and S100A9 mRNA, release of the proteins from the cell and or the signaling/receptors of the homodimers, all of which might be an important goal in managing OA.